

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method for production and purification of a soluble heterologous fusion protein comprising a cellulose binding module (CBM), from transgenic plants or transgenic plant cells expressing said fusion protein, comprising

(a) disrupting the transgenic plant material;

(b) adding an extraction liquid to the plant material, thereby creating a mixture of soluble and insoluble plant material, so as to extract the soluble fusion protein from said disrupted plant material to the liquid phase to obtain a protein extract;

(c) separating the insoluble plant material, comprising cell-wall material and solids, from said protein extract comprising said fusion protein of interest;

(d) contacting said protein extract to a polysaccharide matrix which binds to said fusion protein;

(e) washing the matrix with the bound fusion protein with one or more suitable aqueous solutions; and

(f) eluting the fusion protein from said polysaccharide matrix by adjusting conditions effecting the release of said fusion protein from the matrix,

thereby obtaining the soluble heterologous fusion protein ~~substantially purified~~.

2. (Original) The method of claim 1 wherein said transgenic plant or plant cell is selected from the group of dicotyledonous plants and monocotyledonous plants.
3. (Original) The method of claim 1 wherein said plant cell or transgenic plant is selected from the group of plants including tobacco, rape seed, soy bean, alfalfa, lettuce, barley, maize, wheat, oat and rice.
4. (Currently Amended) The method ~~of any of claims 1-3~~ according to claim 1, wherein the separation step (c) ~~comprises a method is~~ selected from one or more of the group consisting of expanded bed adsorption (EBA), precipitation, filtration, and centrifugation, ~~or any combination thereof.~~
5. (Original) The method of claim 1 wherein affinity binding to said polysaccharide matrix in step (d) comprises a chromatography step.
6. (Original) The method of claim 1, combining steps (c) and (d) in a process step comprising expanded bed adsorption with a polysaccharide matrix, as a measure for simultaneous separation of cell-wall material and solids from said protein extract and affinity binding of said CBM-fusion protein onto the polysaccharide matrix.

7. (Currently Amended) The method of ~~any of claims 1-6~~ claim 1, wherein said conditions effecting the elution of said fusion protein from the matrix are ~~non-denaturing conditions that may be~~ neutral or acidic conditions or ~~involve~~ involving exposure to carbohydrates, or any combination thereof.

8. (Currently Amended) The method of any one of claims ~~[[1-7]]~~ 1, 5, and 6, wherein said polysaccharide matrix comprises cellulose.

9. (Original) The method of claim 8, wherein said cellulose matrix comprises a pharmaceutically compatible cellulose.

10. (Currently Amended) The method of claim 9, wherein said cellulose is ~~Avicel™~~ a microcrystalline cellulose.

11. (Currently Amended) The method of ~~any of claims 1-10~~ claim 1, wherein said transgenic plant or plant cell comprises a nucleic acid sequence encoding for a CBM.

12. (Currently Amended) The method of claim 11, wherein said CBM is heat-stable and remains soluble at ~~elevated~~ temperatures higher than 25 °C.

13. (Currently Amended) The method of claim 12, wherein said ~~region coding for a CBM~~
nucleic acid sequence encoding for a CBM is a coding region of the xylanase10A gene from
Thermotoga maritima.

14. (Currently Amended) The method of claim 13, wherein said coding region ~~encoding~~ for a
CBM comprises a sequence depicted ~~in~~ in SEQ ID NO: 1, or a sequence encoding the same
amino acid sequence or an amino acid sequence with substantial sequence identity to said
sequence.

15. (Original) The method of claim 1, wherein said protein extract is heated to a temperature
in the range of 37°C and 100°C, for a period of time in the range of from 1 min to 120 minutes
during the process.

16. (Currently Amended) The method of claim ~~[[16]]~~ 15, wherein said heated extract is
subjected to the process step comprising expanded bed adsorption with a polysaccharide matrix
for the simultaneous separation of solids and affinity binding of said CBM fusion protein from
the heated extract.

17. (Currently Amended) The method of ~~any of claims 1-16~~ claim 1, wherein said
heterologous fusion protein comprises a protease.

18. (Original) The method of claim 17, wherein said protease is mammalian enterokinase (EK) or an enterokinase active part thereof.

19. (Original) The method of claim 18, wherein said EK comprises a bovine EK catalytic domain (EKc).

20. (Original) The method of claim 19, wherein said bovine EKc is encoded by the nucleic acid sequence shown as SEQ ID NO: 2.

21. (Original) The method of claim 1, wherein said fusion protein comprises a CBM and a heterologous polypeptide of interest intercepted by a proteolytic cleavage site.

22. (New) The method of claim 13, wherein said CBM is the carbohydrate binding domain CBM9-2.